

part of U.S. application Serial No. 13,039, filed February 3, 1993, now U.S. Patent No. 5,480,772.--

In the claims:

Cancel claims 1-86 and substitute therefor the following claims:

87. In transplantation of a donor-cell nucleus other than a sperm or egg nucleus into a recipient egg for the purpose of creating a new organism capable of development, wherein said donor-cell nucleus, due to its gene structure and function, lacks the capacity to give rise to a complete, whole animal if directly transplanted into an activated recipient egg, the improvement comprising incubating said donor-cell nucleus in cytoplasm consisting of two types: first, in cytostatic factor-containing cytoplasm that is arrested in metaphase selected from the group consisting of meiotic metaphase II and mitotic metaphase; and subsequently in activated-egg cytoplasm.

88. The method of claim 87 further comprising step of removing or destroying said recipient egg's own nucleus.

89. The method of claim 88 wherein the step of removing or destroying the recipient egg's own nucleus precedes transplantation.

90. The method of claim 87 wherein the cytoplasm that is arrested in metaphase is a first extract from at least one egg.

91. The method of claim 90 wherein said first extract has been frozen.

92. The method of claim 90 wherein transplantation is by microinjection after the step of incubating with said first extract.

93. The method of claim 90 wherein said first extract is enriched for at least one of the group consisting of mitosis-promoting factor and cytostatic factor.

94. The method of claim 93 wherein said first extract is supplemented with at least one chemical or factor that enhances, stabilizes or extends the life of at least one kinase activity of said extract.

95. The method according to claim 94 wherein said first extract includes an aqueous buffer.

96. The method of claim 87 wherein the activated-egg cytoplasm includes a second extract from at least one egg.

97. The method of claim 96 wherein said second extract has been frozen.

98. The method of claim 97 wherein the cytoplasm that is arrested in metaphase is a first extract from at least one egg, and said first extract has been frozen.

99. The method of claim 96 wherein transplantation is by microinjection after the step of incubating with said second extract.

100. The method of claim 99 wherein said second extract has at least 70% activating capacity as determined by DNA replication.

101. The method of claim 100 wherein said second extract contains at least one inhibitor of an activating extract-promoted event or process.

102. The method of claim 87 wherein the activated-egg cytoplasm includes the recipient egg's cytoplasm.

103. The method of claim 102 wherein the recipient egg is treated so as to prevent activation of its cytoplasm prior to transplantation.

104. The method of claim 87 wherein the cytoplasm that is arrested in metaphase and the activated-egg cytoplasm are from different individuals.

105. The method of claim 104 wherein the different individuals are from different species.

106. The method of claim 87 wherein said donor-cell nucleus is a G2 nucleus.

107. The method of claim 87 wherein said cytoplasm that is arrested in metaphase is supplemented with at least one chemical or factor that enhances, stabilizes or extends the life of at least one kinase activity.

108. The method of claim 87 wherein the cytoplasm is arrested in metaphase and the activated-egg cytoplasm are cytoplasm of different eggs.

109. The method of claim 87 wherein said cytoplasm that is arrested in metaphase is supplemented with an inhibitor of kinase activity.

110. The method of claim 109 wherein said inhibitor is 6-dimethylamino purine (DMAP).

111. The method of claim 87 wherein, prior to contacting the donor-cell nucleus with the cytoplasm that is arrested in metaphase, the cytoskeleton surrounding the donor-cell nucleus is structurally altered.

112. The method of claim 111 wherein said cytoskeleton is structurally altered by at least one reagent selected from the group consisting of a thiol reducing agent, an ionic salt, an anionic chemical, a protease and a chelating agent.

113. The method of claim 111 further comprising step of removing or destroying said recipient egg's own nucleus.

114. The method of claim 113 wherein the step of removing or destroying the recipient egg's own nucleus precedes transplantation.

115. The method of claim 111 wherein the cytoplasm that is arrested in metaphase is a first extract from at least one egg.

116. The method of claim 115 wherein said first extract has been frozen.

117. The method of claim 115 wherein transplantation is by microinjection after the step of incubating with said first extract.

118. The method of claim 115 wherein said first extract is enriched for at least one of the group consisting of mitosis-promoting factor and cytostatic factor.

119. The method of claim 118 wherein said first extract is supplemented with at least one chemical or factor that enhances, stabilizes or extends the life of at least one kinase activity of said extract.

120. The method according to claim 119 wherein said first extract includes an aqueous buffer.

121. The method of claim 111 wherein the activated-egg cytoplasm includes a second extract from at least one egg.

122. The method of claim 121 wherein said second extract has been frozen.

123. The method of claim 122 wherein the cytoplasm that is arrested in metaphase is a first extract from at least one egg, and said first extract has been frozen.

124. The method of claim 121 wherein transplantation is by microinjection after the step of incubating with said second extract.

125. The method of claim 121 wherein said second extract has at least 70% activating capacity as determined by DNA replication.

126. The method of claim 125 wherein said second extract contains at least one inhibitor of an activating extract-promoted event or process.

127. The method of claim 111 wherein the activated-egg cytoplasm includes the recipient egg's cytoplasm.

128. The method of claim 127 wherein the recipient egg is treated so as to prevent activation of its cytoplasm prior to transplantation.

129. The method of claim 111 wherein the cytoplasm that is arrested in metaphase and the activated-egg cytoplasm are from different individuals.

130. The method of claim 129 wherein the different individuals are from different species.

131. The method of claim 111 wherein said donor-cell nucleus is a G2 nucleus.

132. The method of claim 111 wherein said cytoplasm that is arrested in metaphase is supplemented with at least one chemical or factor that enhances, stabilizes or extends the life of at least one kinase activity.

133. The method of claim 111 wherein the cytoplasm is arrested in metaphase and the activated-egg cytoplasm are cytoplasms of different eggs.

134. The method of claim 111 wherein said cytoplasm that is arrested in metaphase is supplemented with an inhibitor of kinase activity.

135. The method of claim 134 wherein said inhibitor is 6-dimethylamino purine (DMAP).

136. The method of claim 87 wherein said activated-egg cytoplasm is treated with a chemical that enhances its nuclear activation activity.

137. The method of claim 136 wherein said chemical is a protein synthesis inhibitor.

138. The method of claim 137 wherein the protein synthesis inhibitor is cycloheximide.

139. The method of claim 87 wherein said activated-egg cytoplasm is treated with a chemical that inhibits at least one event or process promoted by activated-egg cytoplasm.

140. The method of claim 139 wherein said chemical is a phosphodiesterase inhibitor.

141. The method of claim 87 wherein said donor-cell nucleus is the nucleus of a non-dividing cell.

142. A method for activating a nucleus of an animal cell comprising

a) permeabilizing the plasma membrane of said cell, and

b) incubating the nucleus of said cell in cytoplasms consisting of

(i) cytostatic factor-containing egg cytoplasm that is arrested in metaphase selected from the group consisting of meiotic metaphase II and mitotic metaphase, and

(ii) activated egg-cytoplasm,

wherein incubation in said cytostatic factor-containing egg cytoplasm precedes incubation in said activated egg cytoplasm.

143. The method of claim 142 further comprising the step of transplanting said nucleus into a recipient egg.

144. The method of claim 143 wherein said animal cell is a somatic cell.

145. The method of claim 144 wherein said somatic cell is a human somatic cell.

146. The method of claim 144 further comprising the step of destroying said recipient egg's own nucleus.

147. The method of claim 142 wherein said animal cell is a sperm cell.

148. The method of claim 147 wherein said sperm cell is an human sperm cell.

149. The method of claim 147 wherein, prior to incubation in cytostatic factor - containing cytoplasm, the cytoskeleton said nucleus is structurally altered.

150. The method of claim 149 wherein said cytoskeleton is structurally altered by at least one reagent selected from the group consisting of a thiol reducing agent, an ionic salt, an anionic chemical, a protease and a chelating agent.

151. The method of claim 142 wherein said activated-egg cytoplasm is treated with a chemical that enhances its nuclear activation activity.

152. The method of claim 151 wherein said chemical is a protein synthesis inhibitor.